

CLAIMS

1. A modified polypeptide having carbohydrate processing enzymatic activity, said modification comprising substitution of the amino acid residue forming
5 the catalytic nucleophile of an active site by a less nucleophilic amino acid residue, wherein said less nucleophilic residue retains some nucleophilic activity.

2. A polypeptide according to claim 1 comprising an amino acid sequence selected from:

- 10 (a) the amino acid sequence of SEQ ID NO: 2 comprising substitution of the residue E387 by a less nucleophilic residue;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising a substitution at an amino acid residue equivalent to E387 of SEQ ID NO: 2 by a less nucleophilic residue; and
- 15 (c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising a substitution at a position equivalent to E387 of SEQ ID NO: 2 by a less nucleophilic residue

wherein said less nucleophilic residue retains some nucleophilic activity.

20 3. A polypeptide according to claim 1 or 2 wherein said less nucleophilic residue is selected from tyrosine, asparagine, cysteine, glutamine and arginine.

4 The polypeptide according to any one of the preceding claims wherein the polypeptide has glycosyl synthase, glycosyl hydrolase and/or transglycosylase
25 activity.

5. The polypeptide according to any one of the preceding claims wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus* β -glycosidase.

30 6. A polypeptide according to any one of the preceding claims which further comprises one or more mutations selected to broaden the substrate specificity of the polypeptide compared to a polypeptide not so modified.

7. A polypeptide according to claim 6, wherein said mutation(s) are selected from:

- 5 (a) at least one of W433, E432 and M439 of the amino acid sequence of SEQ ID NO:2;
- (b) at least one amino acid residue equivalent to W433, E432 or M439 of SEQ ID NO: 2 in the amino acid sequence of a family 1 glycosyl hydrolase, and
- (c) at least one amino acid mutation at a position equivalent to W433, E432 or M439 of SEQ ID NO: 2 in a variant of (a) or (b) having carbohydrate
10 processing enzymatic activity.

8. The polypeptide according to claim 7 in which the polypeptide comprises:

- 15 (i) SEQ ID NO: 2 having one or more of W433, E 432 and M439 substituted by cysteine, valine or alanine; or
- (ii) the amino acid sequence as defined in (b) or (c) having one or more of the amino acid residues equivalent to W433, E432 or M439 substituted by cysteine, valine or alanine.

20 9. A polynucleotide encoding a polypeptide having carbohydrate processing enzymatic activity according to any one of the preceding claims.

10. An expression vector comprising a polynucleotide according to claim 9.

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11. A host cell transformed with a vector according to claim 10.

12. A method for hydrolysing a β -glycoside, synthesising a β -glycoside or transglycosylation, which method comprises contacting a glycoside substrate with
30 a modified polypeptide as defined in any one of claims 1 to 8.

13. The method according to claim 12 wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside and a glucuronide.
- 5 14. The method according to claim 12 wherein the polypeptide is contacted with a sample containing at least two different glycosides.